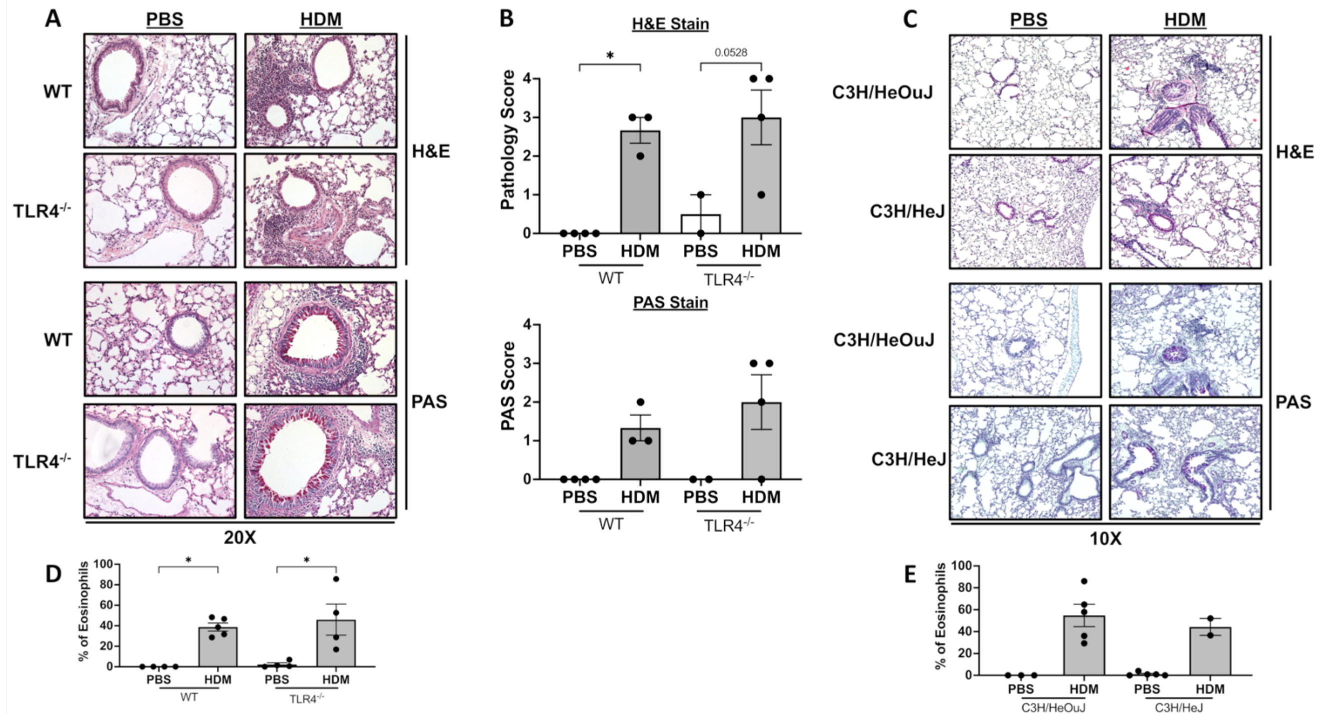

Figure S1: TLR4 expression is not a requirement for allergic inflammation after treatment with HDM.

Lung sections from WT (C57BL/6J), TLR4$^{-/-}$, C3H/HeOuJ, and C3H/HeJ mice (n=4 mice/treatment group) were isolated on day +17 and were fixed, blocked, and then stained with Hematoxylin & Eosin or a Periodic Acid Schiff Stain to determine allergic inflammation at 20X (A) and 10X (C) lens magnifications. WT and TLR4$^{-/-}$ lung sections were then evaluated by a pathologist blinded to the experimental conditions and scored on a scale of 0-4 (B). Histological images are from 2 independent trials. Scoring is from 1 trial with each point representing an individual mouse. Lungs of (D) WT (C57BL/6J), (D) TLR4$^{-/-}$, (E) C3H/HeOuJ, and (E) C3H/HeJ were lavaged with 1 mL of PBS to obtain BAL and was used for cell counts by Cytospin followed by differential counting to calculate the (D, E) percentage of eosinophils (n=2-5 mice/treatment group). All data was analyzed by 2-way ANOVA with Tukey's Multiple Comparisons Test: *, $P<0.05$. Error bars represent mean ± SEM.

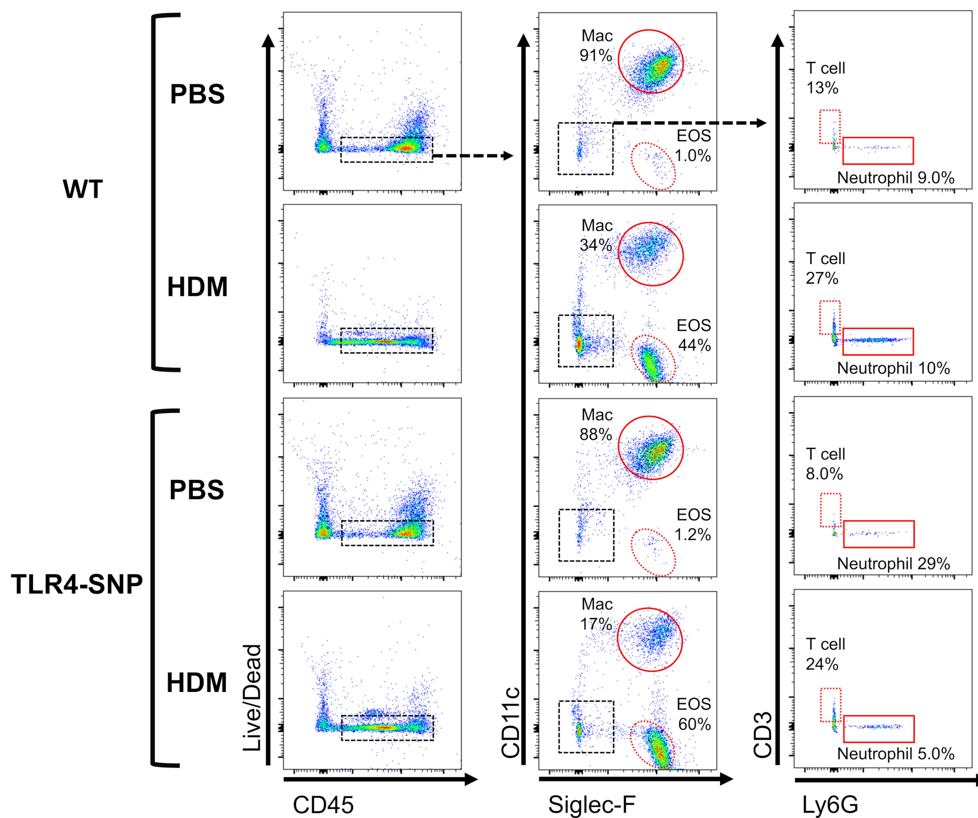

Figure S2: Gating strategy for phenotypic characterization of BAL cells.

On day +17, all BAL samples were isolated and prepared for flow cytometry. Samples were first stained with a LIVE/DEAD Fixable Stain (Invitrogen), blocked with TruStain FcX (anti-mouse CD16/32 Ab, Biolegend), and then immediately stained for cellular surface markers with fluorphore-conjugated antibodies against CD45, Siglec-F, CD11c, CD3, Ly6G. Stained BAL cells were analyzed using a LSRFortessa Cell Analyzer (Becton Dickinson). Cells were first gated on Live, $CD45^+$ Lymphocytes, and then separated into eosinophils ($CD45^+$,Siglec-F^+,$CD11c^-$) and macrophages ($CD45^+$,Siglec-F^+,$CD11c^+$). The Siglec-F^-,$CD11c^-$ population further gated using CD3 and Ly6G. T cells were identified as $CD45^+$, $CD3^+$,$Ly6G^-$,Siglec-F^-,$CD11c^-$ and Neutrophils were identified as $CD45^+$, $Ly6G^+$,$CD3^-$,Siglec-F^-,$CD11c^-$. Shapes indicate the percentage of cells that are within that gate.